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T-Cell Vaccination in Autoimmune Diseases From Laboratory to Clinic

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ABBREVIATIONS

APC	antigen-presenting cell
DTH	delayed-type hypersensitivity
EAE	experimental autoimmune encephalomyelitis
MBP	myelin basic protein
MHC	major histocompatibility complex
MS	multiple sclerosis
PCR	polymerase chain reaction
PHA	phytohemagglutinin
RA	rheumatoid arthritis
TcR	T-cell receptor

INTRODUCTION

Pathogenic autoreactive T cells are viewed as pathogens in the induction of T-cell-mediated experimental autoimmune diseases. When rendered avirulent, they can be used as vaccines to prevent and treat the diseases, in analogy with traditional microbial vaccination against infectious agents [1]. Administration of autoreactive T cells as vaccines induces or augments the regulatory networks specifically to suppress the eliciting autoreactive T cells. On one hand, T-cell vaccination provides a unique tool to study in vivo network regulation potentially operative in the normal immune system to control autoreactive T cells. Studies in this regard are beginning to provide new insights into the mechanisms by which autoreactive T cells are regulated in health and an aberrant regulation that may underlie autoimmune process. On the other hand, T-cell vaccination provides a potential therapeutic option for human autoimmune pathologies to target and deplete autoreactive T cells involved in the diseases' pathogeneses. Preliminary indications from these clinical studies have generated great enthusiasm that T-cell vaccination or T-cell receptor (TcR) peptide vaccination may be useful in treatment of certain human autoimmune diseases, such as multiple sclerosis (MS) and rheumatoid arthritis (RA).

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In this report, recent advances regarding the mechanism of T-cell vaccination are summarized with recent data from our laboratory to integrate current information on its clinical applications in human autoimmune diseases.

PATHOLOGIC ROLE OF AUTOREACTIVE T CELLS IN AUTOIMMUNE PATHOGENESIS

Not all autoreactive T cells are deleted in the thymus, in contradiction with the clonal selection paradigm [2]. Those T cells with the receptors for a broad spectrum of self-antigens represent part of the normal T-cell repertoire and naturally circulate in the periphery [3]. It remains unresolved why autoreactive T cells are allowed, during their evolution, to undergo differentiation in the thymus and are accommodated in the periphery. While their physiologic role is not understood, these autoreactive T cells, when activated, present a potential risk in the induction of autoimmune pathologies. Autoreactive T cells can be isolated from normal individuals without the consequences of autoimmune diseases. It has been established that antigen recognition of autoreactivity by itself is not sufficient to mediate the autodestructive process. One of the prerequisites for autoreactive T cells to be pathogenic is that they must be activated, as evidenced in experimental autoimmune encephalomyelitis (EAE). EAE can be induced actively in susceptible animals by injecting myelin basic protein (MBP) emulsified in an adjuvant or passively by injecting MBP-reactive T-cell lines and clones derived from MBP-sensitized animals [4]. When activated in vitro, very small numbers

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of MBP-reactive T cells are required to induce EAE, while 100-fold more resting T cells with the same reactivity are incapable of mediating the disease. There are a number of potential mechanisms by which circulating autoreactive T cells are accidentally activated and undergo clonal expansion. In addition to antigenic stimulation, autoreactive T cells may be activated through several pathways that are potentially associated with viral or bacterial antigens.

One of the mechanisms involves superantigens that interact with TcR Vβ gene products and the major histocompatibility complex (MHC) class II molecules in an unconventional manner to activate large portions of the T-cell repertoire. Autoreactive T cells bearing certain TcR Vβ products can be selectively activated by bacterial superantigens during the course of a bacterial infection, offering an explanation for oligoclonal expansion of autoreactive T cells compartmentalized in the affected organs in both RA and MS [5–7]. Recent data from our laboratory indicate that a number of bacterial superantigens are capable of activating human T-cell clones reactive to MBP, a presumed autoantigen in MS, dependent on their TcR Vβ gene usage. Other proposed mechanisms include molecular mimicry in which a bacterial or viral antigen sharing molecular homology with an autoantigen provokes immune responses against self-tissue [8].

However, not all activated autoreactive T cells will necessarily undergo clonal expansion to a certain extent that may lead to autodestructive responses. This process is normally controlled by regulatory systems that govern and prevent clonal expansion of activated autoreactive T cells in the periphery. There is evidence indicating that such regulatory networks are operating in vivo. An aberrant regulation is postulated to lead to clonal expansion of autoreactive T cells and a destructive autoimmune process, a common pathologic feature shared by autoimmune diseases [9].

A recent example is illustrated in MS, an inflammatory disease of the central nervous system. The disease is pathologically characterized by focal infiltration of T cells and macrophages into the brain, followed by demyelination. The T-cell responses to MBP are predominantly directed at a few immunodominant regions of MBP and these MBP-specific T cells occur at a relatively low frequency in peripheral blood of patients with MS and healthy individuals [10]. The finding by itself may not coincide with current hypothesis in which the inflammation in the MS brain is postulated to involve, at least in a primary stage, the T-cell responses to MBP. However, when further examined for their activation state by interleukin-2 stimulation, a quite different pattern emerged, suggesting these MBP-reactive T cells have undergone an activation process in vivo in patients with MS, as opposed to normal individuals. The activated MBP-reactive T cells account for even higher proportions of T cells residing within the brain compartment, indicative of a specific accumulation of the autoreactive T cells in the affected organ [11]. The activation and clonal expansion of autoreactive T cells in MS are in accordance with recent studies that demonstrate the oligoclonal nature of T cells confined to cerebrospinal fluid in MS patients [6]. Oksenberg et al. [12] recently provided further evidence suggesting that MBP-reactive T cells accumulated within the MS lesions have undergone a clonal expansion, as demonstrated by limited motifs within the TcR Vβ CDR3 region (the third complementary determining region) of the T cells derived from the lesions. These findings have provided an important rationale for setting up clinical trials in MS, using T-cell vaccination or a TcR peptide corresponding to a target sequence in the TcR.

IN VIVO IMMUNE REGULATION OF AUTOREACTIVE T CELLS

A number of mechanisms are operative in vivo to regulate autoreactive T cells through processes that are largely not understood. Such mechanisms may involve T-cell clonal anergy, which causes autoreactive T cells to be unresponsive and due to inappropriate antigen presentation and a signaling defect in the T-cell activation pathways, tolerant to an antigenic stimulation. It has been shown that human MBP-reactive T cells are anergized upon encountering free MBP peptides without professional antigen-presenting cells (APCs) [13]. Another important regulatory mechanism involves clonotypic networks that regulate autoreactive T-cell clones by interacting with their clonotypes. Hypervariable determinants within the TcR variable regions, including the junctional regions of both α and β chains, constitute the clonotype that uniquely marks an individual autoreactive T-cell clone and is recognizable by its regulators. Thus, autoreactive T cells are regulated at a clonal basis to ensure the "fine-tuning" of the regulatory system in order to ignore unrelated T cells. On the other hand, immune responses to the framework regions generally conserved among T cells are tolerant during the evolution of the immune system, since their sequences are germline encoded.

There is increasing evidence that the clonotypic regulatory network plays a central role in controlling autoreactive T cells. Clonal activation and expansion of autoreactive T cells signals the network to suppress activated autoreactive T cells, a specific event that spares other unrelated T cells in the immune system. This regulatory network preexists as part of the normal T-cell repertoire and has been shown to regulate autoreactive T cells

naturally [14]. The mechanisms regarding the signaling molecules on target T cells that elicit the clonotypic interactions are still not understood, but are thought to involve both CDR2 and CDR3 hypervariable regions of the TcR Vβ chain. This is strongly supported by recent observations that immunization with a peptide corresponding to the CDR2 region of encephalitogenic T cells in experimental animals results in protection against EAE [15, 16]. A similar regulatory mechanism has been proposed to control MBP autoreactive T cells in humans [17].

Many autoimmune diseases are thought to result from an unregulated expansion of pathogenic autoreactive T cells. An improper function of the network may lead to an aberrant regulation that may in turn be responsible for activation and hyperactivity of autoreactive T cells. Thus, it is important to identify a potential signaling defect(s) within the regulatory system in autoimmune diseases so that a therapeutic approach may be developed to correct the error and upregulate the system. The following sections are devoted to discussion of the recent experimental evidence in this regard and related issues emerging from recent clinical trials of T-cell vaccination in MS and RA.

CLONOTYPIC REGULATION BY T-CELL VACCINATION

Pioneering work by Irun Cohen and coworkers has shown that T-cell vaccination induces clonotypic network interactions to produce therapeutic effects in preventing and treating experimental autoimmune diseases. It was first studied in EAE in Lewis rats and later in other experimental autoimmune diseases models, including adjuvant arthritis and autoimmune thyroiditis [1, 18, 19]. Pathogenic autoreactive T cells, capable of inducing an autoimmune disease, can be rendered avirulent by attenuation and can be administered as vaccines to prevent subsequent induction of the disease. These studies have established that vaccine T cells must be activated and attenuated, the two prerequisites for vaccine T cells to be effective in T-cell vaccination. The methodologies used to attenuate vaccine T cells vary from irradiation to pressure treatment and chemical cross-linking. Despite some discrepancies in the efficiency, all of these methods seem to be effective in inducing a protective response.

The disease resistance induced by T-cell vaccination is long-lasting and can be adoptively transferred to a naive receipient by regulatory T cells of both CD4 and CD8 phenotypes reactive to the immunizing T cells [20]. At least two types of regulatory T cells have been identified so far to contribute to the disease resistance. Anticlonotypic T cells, as the major regulators, recognize the clonotypes of the target TcR. The protection induced by T-cell vaccination is potentially mediated through direct killing of the target T cells by anti-idiotypic T cells, or through other inhibitory mechanisms [20]. It has been suggested, however, that the TcR may not be the only protective element to modulate autoreactive T cells. Other regulatory T cells are also important, in concert with the anti-idiotypic response, in suppressing autoreactive T cells by interacting with cellular markers other than the TcR clonotypes. One example is the regulatory T cells identified as "anti-ergotypic T cells," which respond not to the TcR but to a marker of their state of activation. These anti-ergotypic T cells are shown to contribute partially to the disease resistance induced by T-cell vaccination [21].

It is reasonable to propose that T-cell vaccination may act through the same regulatory pathways preexisting and naturally operating in the periphery. In the normal immune system, the regulatory mechanisms, including the clonotypic networks, may be developed to accommodate and regulate a limited amount of circulating autoreactive T cells within a physiologic threshold, which may not be able to cope with an acute pathologic dose of autoreactive T cells typically used to induce EAE. By the same token, preinoculation with attenuated encephalitogenic T cells upregulates and directs the networks toward an active mode capable of suppressing subsequent administration of encephalitogenic T cells to prevent the disease.

The mechanism potentially underlying T-cell vaccination is not completely understood. It is inconclusive as to the molecular identity of the target epitopes within the TcR recognized by the anticlonotypic T cells. Experimental evidence accumulated to date from animal experimentation indicates that the anti-idiotypic T cells recognize the clonotypic structure within the TcR, most likely within the immunogenic regions, including the CDR2 and the CDR3 regions. However, it is deduced indirectly from a series of experiments indicating that anti-idiotypic T cells respond to the immunizing T cells with a given antigen specificity and pathogenic T cells only protect the disease that they are able to induce. For example, vaccination with MBP-specific T cells only prevents the induction of EAE but does not protect adjuvant arthritis that is mediated by T cells reactive to *Mycobacterium tuberculosis*, and vice versa. However, the data do not necessarily identify the target sequence responsible for triggering the specific anti-idiotypic response.

Recent evidence from the TcR peptide vaccination provides some indications for the involvement of the CDR2 and CDR3 (V–D–J junctional) regions of the TcR Vβ chain in eliciting the anticlonotypic response. Vandenbark and Howell and coworkers [15, 16] demonstrated successful prevention of EAE by inoculating

Lewis rats and mice with a peptide corresponding to the CDR2 region of the target TcR. The anti-idiotypic T cells isolated from the vaccinated animals are capable of conferring protection to the syngeneic naive recipients by adoptive transfer. These observations support the view that the clonotypic epitopes are immunodominant regions that elicit anticlonotypic T-cell responses in T-cell vaccination. However, it is unlikely that the anticlonotypic T-cell recognition of the CDR2 peptide alone accounts for the protective effect seen in T-cell vaccination. This is supported by the observations that a higher dose of the CDR2 peptide is required to achieve the protection against EAE and that only activated T cells are effective in T-cell vaccination.

Therefore, other mechanisms are likely involved in contributing to effective protection. These mechanisms are potentially associated with the functional and cellular characteristics of the vaccine T cells, such as their activation markers and cytokine profile, which are only available in vaccine T-cell preparation. In addition to T-cell activation markers involved in triggering anti-ergotypic responses, the role of cytokine production of the vaccine clones has been proposed recently by W. Van Eden and coworkers (personal communication). They observed that an arthritogenic T-cell clone (A2b) capable of inducing adjuvant arthritis differs only in its cytokine profile from a syngeneic protective T-cell clone (A2c) and shared the same antigen reactivity and sequence homology within the TcR variable regions. Furthermore, Saruham-Direskeneli and coworkers [17] reported recently that anticlonotypic T cells can be isolated by in vitro stimulation of T cells with synthetic peptides of both CDR2 and CDR3 regions of an MBP-specific T-cell clone [17]. These anticlonotypic T-cell lines express the CD4 phenotype and are cytotoxic against the MBP-specific T-cell clone bearing the target CDR2 and CDR3 regions. Again, these data support but do not necessarily provide direct evidence as to whether and how the CDR2 and CDR3 regions, in their assembled form on the target cell surface, are recognized by anticlonotypic T cells.

TARGETING OF AUTOREACTIVE T CELLS AS AN IMMUNOTHERAPEUTIC APPROACH FOR HUMAN AUTOIMMUNE DISEASES

To develop a specific immunotherapy for autoimmune diseases in general, the mechanism underlying the pathogenesis of the disease regarding the pathogenic autoantigen and autoreactive T cells that mediate the autoimmune process leading to the diseases' pathogenesis should be defined. Thus, an immunotherapy to target and eliminate these pathogenic T cells can be designed. These studies are largely guided by experimental animal models. The subunit of the T cells that distinguishes the pathogenic T cell from unrelated T cells is the TcR. The TcR seems to be the most appropriate target structure in designing an effective and specific immunotherapy. To be successful, an obvious requirement for targeting of the TcR is that the pathogenic T-cell population must be homogeneous regarding the restricted TcR repertoire for recognizing autoantigens in question. This condition seems to be met in EAE, where encephalitogenic MBP-reactive T cells are restricted to very limited epitopes on MBP and the TcR Vβ gene segments in the induction of the disease in Lewis rats and PL/J mice [22–24]. These restrictions in the diversity of the pathogenic T-cell responses permit specific immune intervention. Various therapeutic strategies have been designed accordingly to target the TcR by monoclonal antibodies to the Vβ gene product, preferentially used by encephalitogenic T cells and by vaccination with a peptide matching the CDR2 region of the responsible Vβ gene. These studies have shown remarkable success in preventing the development of EAE in sensitized animals [16, 17, 24].

Some of these studies have even been extended to human autoimmune diseases. For instance, a peptide corresponding to TcR Vβ 5.2 is being used in a clinical trial to treat patients with MS and a Vβ 14 peptide is being used to vaccinate patients with RA. The clinical trials are based upon the authors' experiments, suggesting that a single or limited TcR Vβ gene is preferentially used by MBP-specific T cells in MS [25] and the oligoclonal T cells derived from synovial fluid of RA [7]. However, in the case of MS, our study and a number of other independent studies published and to be published do not support a preferential use of TcR Vβ 5.2 or Vβ 6.1 among MBP-specific T-cell clones isolated from patients with MS [25–27]. Rather, MBP autoreactive T-cell clones showed a heterogeneous pattern of the TcR Vβ gene usage that is relatively restricted in individuals. Thus, it would significantly impair the feasibility of using such an approach to eliminate pathogenic autoreactive T cells therapeutically.

In addition to a heterogeneous TcR Vβ gene usage among MBP-reactive T cells, there is no clear evidence for a pathogenic epitope directly associated with the autoimmune process in most autoimmune diseases studied to date. Returning to MS as an example, T-cell reactivity to the two immunodominant epitopes, the 84–104 and the 143–168 regions, dominates the responses to MBP. The preferential T-cell recognition of an immunodominant epitope(s) varies among individuals, irrespective of the presence of the disease (reviewed in Zhang et al. [28]). These findings would argue against a direct role of T-cell responses to the MBP epitopes. Rather, it is indicative of their immunodominant nature in human

responses to MBP. The complexity is further increased by the undefined autoimmune process responsible for the diseases' pathogenesis in humans, which is not necessarily comparable with the underlying disease process in EAE. For example, both RA and MS are chronic relapsing diseases whose clinical courses differ substantially from most experimentally induced animal models. This is illustrated among others by an inconstant cellular dominance of heterogeneous effectors at the lesion sites during different disease stages. Autoreactive T cells are thought to play a central role in the early lesions, which may be taken over, in more chronic lesions, by inflammatory cells recruited during the secondary events. Hence, in most cases, it is not necessarily clear which element(s) of autoreactive T cells should be targeted and when they should be targeted to produce clinical benefits.

In this regard, without knowledge of a clearly distinguishing cellular marker of disease-related autoreactive T cells, a specific immunotherapy may take advantage of T-cell vaccination, which uses all of the pathogenic autoreactive T cells and a natural regulatory pathway specifically to regulate pathogenic autoreactive T cells. In this case, the immune system is upregulated to define a relevant target epitope(s) within the TcR and additional cellular markers, in their naturally assembled form, to achieve an adequate regulatory response.

PRELIMINARY INDICATIONS OF CURRENT CLINICAL TRIALS OF T-CELL VACCINATION

The successful demonstration of T-cell vaccination in the treatment of organ-specific autoimmune diseases in animal experimentation has led to great enthusiasm that human autoimmune diseases may be amenable to treatment in a similar fashion.

An initial clinical trial by Hafler and coworkers [29] was carried out in four patients with MS. Patients were inoculated with formaldehyde-fixed autologous T-cell clones isolated by phytohemagglutinin (PHA) stimulation from cerebrospinal fluid. The T-cell clones used for vaccination were chosen based upon their phenotype (CD4+), growth characteristics, and the expression of dominant rearranged TcR genes that represent the oligoclonal T cells harbored in the brain compartment. The study revealed some interesting immunologic findings regarding a general inhibition of T-cell stimulation via CD2 pathway and increases in autologous mixed-lymphocyte responses after vaccination. Because the antigen specificity of these T cells was undefined at the time, it was unclear whether inoculation with the cerebrospinal fluid-derived T cells downregulated a related autoreactive T-cell population. Two other observations related to similar attempts in RA were reported by Van Laar and his associates [30]. In one case, synovial T cells derived from patients with RA were used, based on the assumption that the cell preparation might contain pathogenic T cells activated by a putative autoantigen(s) within the synovial compartment. The inoculates were polyclonally activated and expanded, in the absence of accessory cells, by an immobilized anti-CD3 antibody. Attenuated T cells, 50×10^6, were injected subcutaneously into a group of 13 patients. In another case, T-cell lines reactive to nickel were used for vaccination in nickel-sensitized donors to assess immunologic responses to the inoculates (personal communication). In both cases, however, no significant immunologic responses were observed, as opposed to the prevaccination values, with respect to T-cell response to the inoculates and delayed-type hypersensitivity (DTH) reaction. In a separate in vitro study, the same authors demonstrated T-cell response to activated synovial T cells reactive with *Mycobacterium* in an unvaccinated patient with RA [31].

On the basis of the potential pathologic role of MBP-specific T cells in the pathogenesis of MS, and especially encouraged by the successful treatment of EAE by T-cell vaccination, we recently vaccinated a group of six MS patients with irradiated MBP-specific T-cell clones. The T-cell clones were selected based upon their reactivity to the two immunodominant regions of human MBP, which dominated T-cell responses to MBP in these individuals. Other supporting evidence for selecting T-cell clones with this peptide reactivity comes from a study demonstrating that MBP-reactive T cells isolated from cerebrospinal fluid of MS patients display the same peptide reactivity to the two immunodominant epitopes as the T-cell clones derived from peripheral blood [11].

Our data obtained from current clinical trials provide the first experimental evidence that inoculation with irradiated MBP-reactive T-cell clones induces a proliferative response to the vaccine clones. This response is accompanied by a marginal reactivity to the nonspecific autologous T cells generated by PHA. Another important observation of our study is the progressive decline and an eventual depletion of circulating MBP-specific T cells, which corresponds reciprocally with the proliferative response to the vaccine clones, as illustrated in Fig. 1. The results suggest that the depletion of circulating MBP-specific T cells is induced by T-cell vaccination and is an antigen-dependent event [32]. Figure 2 outlines the protocol and schemes that we used, which differ in many technical details from the previous clinical trials. The procedure used in our study seems to be effective in the induction of a specific immunologic response to the immunizing T-cell clones that specifically depletes circulating MBP-reactive T cells in patients with MS.

The anticlonotypic T-cell lines and clones of both

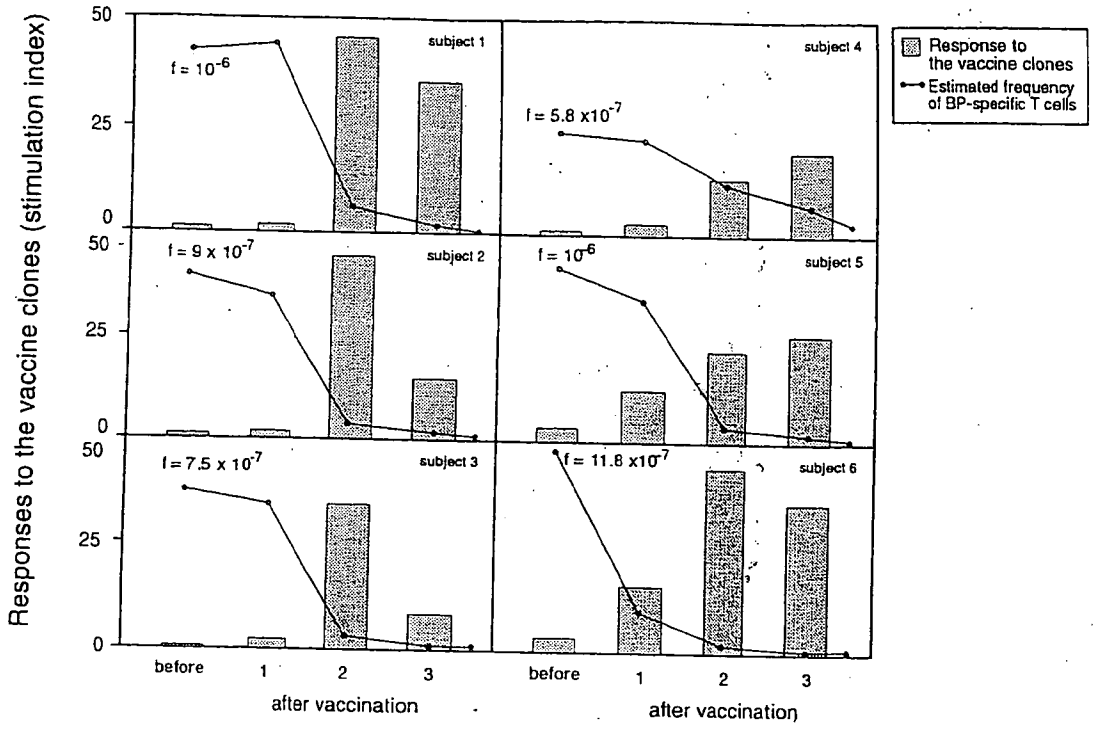

FIGURE 1 The proliferative T-cell responses to the vaccine clones and changes in the estimated frequency of circulating MBP-reactive T cells in six patients with MS, before and after each inoculation. The responses to the vaccine clones were determined in proliferation assays, following the schemes illustrated in Fig. 3, in which peripheral blood mononuclear cells (PBMCs) were cultured with irradiated vaccine clones. The proliferative responses were calculated as stimulation indices (proliferation of PBMCs in the presence of vaccine clones per the sum of spontaneous proliferation of PBMCs alone and residual proliferation of irradiated vaccine clones). Data are given as mean stimulation indices of seven assays after each inoculation. The frequency of MBP-reactive T cells was estimated according to the method described in Zhang et al. [10]. The frequency before vaccination is indicated on the *lines*, which ranges from 5.8×10^{-7} to 11.8×10^{-6} in these patients.

CD4 and CD8 phenotypes generated from recipient patients display a specific clonotypic recognition of the clones used for vaccination. The CD8+ anticlonotypic T-cell clones are capable of lysing the vaccine clones in an MHC class-I-restricted manner. Our current experiments on defining the target elements recognized by the anticlonotypic T cells suggest at least two recognition patterns. One potentially involves the V–D–J junctional region of the β chain or the V–J region of the α chain, as indicated by the recognition of anticlonotypic T cells to a target TcR sequence uniquely expressed on the immunizing T cells. The anticlonotypic T-cell clones of this recognition pattern responded specifically to the immunizing MBP-specific T-cell clone but not to a total of 18 other autologous and allogeneic MBP-specific T-cell clones, not used for vaccination, with defined peptide reactivity and TcR V gene usage. The other pattern relates to a clonotypic marker relatively conserved within the Vα region among autologous T cells. They are characterized by the reactivity, in addition to the immunizing T-cell clones, to other MBP-specific T cells bearing the same or related Vα gene products. Our polymerase chain reaction (PCR) analysis of peripheral lymphocytes collected at different time points before and after each vaccination revealed a depletion of T cells bearing the same or related Vα products as the vaccine clones. The anticlonotypic T cells with both recognition patterns are effective in depleting the immunizing T cells, as evidenced by complete elimination of circulating MBP-reactive T cells in all six recipients. However, one potential drawback to the second pattern of clonotypic regulation is the limited specificity for target MBP-reactive T cells, as the clonotypic responses may also be directed at other T cells bearing the same clonotypes, regardless of their antigen specificity. Figure 3 illustrates the potential involvement of the antigenic regions within the TcR as recognition signals to elicit the anticlonotypic T cells.

CONSIDERATIONS FOR FUTURE CLINICAL STUDIES

The preliminary clinical trials studied to date indicate that T-cell vaccination is technically feasible and causes

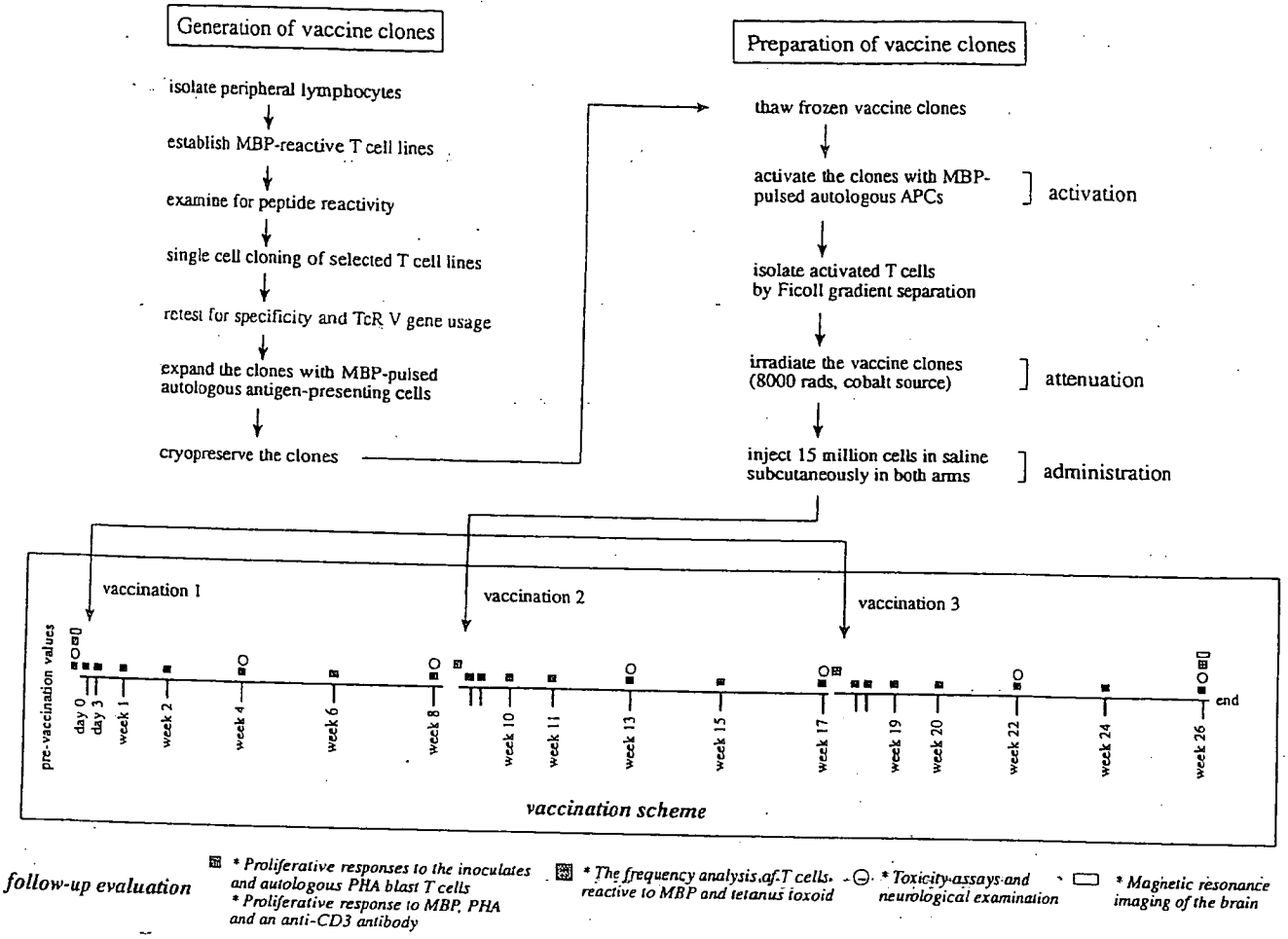

FIGURE 2 Schematic illustration of the technical procedure and immunization scheme used in the clinical trial.

no toxic or untoward side effects in humans. It seems plausible, from our experience in the MS clinical trials, to target and deplete a population of autoreactive T cells involved in the autoimmune process. Since all of these phase-1 studies were not designed to evaluate the clinical efficacy in both MS and RA, it was not at all clear whether T-cell vaccination in these cases is clinically beneficial. Although no clear remission is observed, preliminary indications suggest some clinical improvement as to reduced disease scores in the very small numbers of recipients in the RA trial and our MS trial. Autoimmune mechanisms are highly complex, however, and involve heterogeneous effector cell populations at different stages of the disease process. For example, in MS, activated MBP-specific T cells are thought to be involved in the initiation of autoimmune process in the brain, which subsequently recruits other inflammatory cells, such as γδ T cells and macrophages, partially by producing inflammatory cytokines. Thus, demyelination is mediated by these inflammatory cells and cytokines in the secondary events in which MBP-specific T cells may not necessarily play a direct role. If the exacerbation of chronic progressive MS is not directly mediated by MBP autoreactive T cells, T-cell vaccination may not improve the clinical course of the disease, since the responses are unlikely to dispose of the inflammatory cells accumulated at the lesions and unlikely to reverse the myelin damage. This may explain the lack of significant clinical improvement regarding the disease score and magnetic resonance imaging of the brain lesions in the chronic progressive patients in our vaccination study. Therefore, in order to produce clinical benefits, T-cell vaccination may need to be applied at an earlier stage of the disease when pathogenic T cells dominate initial inflammatory processes. Under these considerations, our second-phase study is designed to evaluate the clinical aspects of T-cell vaccination, aiming at patients with remitting–relapsing characteristics and with acute brain lesions.

Several issues need to be addressed as we apply this approach in future clinical trials regarding the mode of

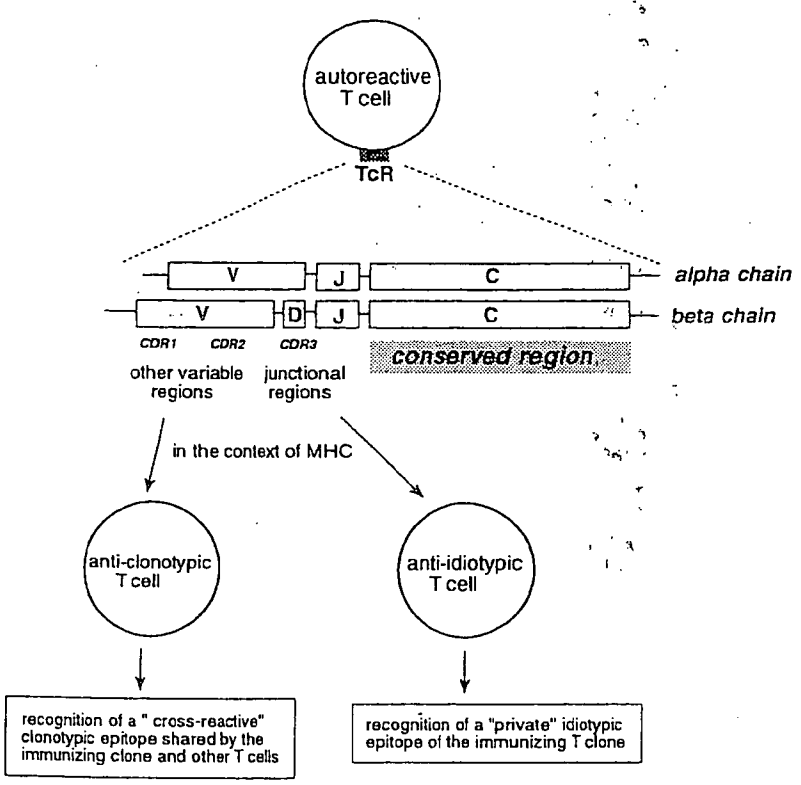

FIGURE 3 The TcR gene organization and the specific sites encoding the variable and junctional regions within the target TcR potentially eliciting the anticlonotypic T-cell responses. Recognition of anti-idiotypic T cells to the junctional regions of the target TcR elicits a specific depletion/inhibition of the immunizing T-cell clone. Anticlonotypic T cells with the recognition pattern to a "cross-reactive" clonotype within the variable regions of the α chain and β chain may deplete/inhibit, in addition to the immunizing clone, other T-cell clones sharing the same clonotype.

attenuation, immunization scheme, route of injection, and an optimal system to measure immunologic responses. First, the selection of autoreactive T-cell clones has an obvious importance in focusing the immune attack on pathogenic T cells to spare unrelated T cells. Ideally, the T-cell clones selected for vaccination should carry an idiotypic or clonotypic epitope(s) characteristic of targeted autoreactive T cells. The target idiotypic determinants are characteristically reflected by the reactivity of autoreactive T cells to a pathogenic or immunodominant epitope(s) of a given autoantigen. In MS, for example, T-cell clones reactive with the two immunodominant regions are relevant targets since they carry the idiotypic epitopes that dominate autoreactive T-cell responses to MBP. In cases where pathogenic autoreactive T cells use a single or a limited TcR V gene(s), T cells bearing this preferentially used V gene product(s) may be selected for vaccination. This may result, however, in depletion of a subset of T cells expressing the target V gene product(s) shared by other T cells.

In addition to the selection of relevant vaccine clones, the way that the T cells are activated may also affect their effectiveness in T-cell vaccination. Activation of vaccine T cells by both antigenic and mitogenic stimulation in the presence of antigen-presenting/accessory cells has been shown to induce effectively the protective response in vaccinated animals. The accessory cells seem to deliver additional signals required to equip the vaccine T cells fully to be effective. This is suggested by the inability of vaccine T cells activated with interleukin 2 alone, in the absence of accessory cells, to induce the protective effect (Irun Cohen, personal communication). Furthermore, the immunization scheme presents another important factor in determining the effectiveness of the vaccination. T-cell vaccination follows the same boosting principle as microbial vaccination to enhance an effective response. Our experience with T-cell vaccination with irradiated MBP-specific T-cell clones suggests the essential requirement of multiple inoculations (at least two inoculations) in order to achieve a substantial anticlonotypic response.

The mode of attenuation may influence the immunogenicity of the vaccine clone in eliciting the anticlonotypic response. The successful induction of anticlonotypic response by using irradiated MBP-specific T cells favors our view that while irradiation effectively attenuates T cells, it preserves major physiologic features of the cell surface markers, most importantly the TcR molecules, and the membrane stability. These features are important to render vaccine T cells recognizable in the same manner as they are seen by the immune system in vivo. Chemical fixation with formaldehyde or related methods alters the cell membrane, however, and probably gives rise to unpredictable structural changes, di-

rectly or indirectly affecting the way that the TcR molecules are recognized. The induced cell surface changes may not necessarily enhance the desired immunogenecity.

Finally, we remain hopeful that MBP-specific T-cell clones isolated from the peripheral blood as well as cerebrospinal fluid of MS may display a limited motif within the V–D–J junctional region of the β chain, as suggested by Oksenberg et al. [12]. Further clarification of the target sequences recognized by anticlonotypic T cells may use the advantages of TcR peptide vaccination to design a peptide corresponding to a predominant CDR3 sequence motif characteristic for pathogenic autoreactive T cells.

In conclusion, what we are learning from T-cell vaccination promises to shape our view of immune regulation both in health and in disease. Its therapeutic application in human autoimmune diseases will provide an alternative to the final treatment.

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